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(54) Interleukin-1 receptor antagonist beta (il-1rabet)

Interleukin-1 Rezeptor Antagonist beta (il-1rabet)

Antagoniste beta d'un récepteur de l'interleukine-1 (il-1rabet)

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(56) References cited:
WO-A-96/09323

- EMBL Databank, Databank entry HS162193,
Accession number G10162, 17/08/1995, Murray
J. et al., Cooperative Human Linkage Center
XP002064218**

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Description**FIELD OF INVENTION**

5 [0001] This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Interleukin-1 family, hereinafter referred to as IL-1ra beta. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

10 BACKGROUND OF THE INVENTION

[0002] Interleukin 1 refers to two proteins (IL1 α and IL1 β) which play a key role early in the inflammatory response [for review see C.A.Dinarello, Blood, 87:2095-2147 (1996) and references therein]. Both proteins are made as 31kDa intracellular precursor proteins which are cleaved upon secretion to yield mature carboxy-terminal 17kDa fragments which are biologically active. In the case of IL-1 β , this cleavage involves an intracellular cysteine protease, known as ICE, which is required to release the active fragment from the inactive precursor. The precursor of IL-1 α is active.

[0003] These two proteins act by binding to cell surface receptors found on almost all cell types and triggering a range of responses either alone or in concert with other secreted factors. These range from effects on proliferation (eg of fibroblasts, T cells), apoptosis (eg A375 melanoma cells), cytokine induction (eg of TNF, IL1, IL8), receptor activation (eg E-selectin), eicosanoid production (eg PGE2) and the secretion of degradative enzymes (eg collagenase). To achieve this, IL-1 activates transcription factors such as NF-KB and AP-1. Several of the activities of IL-1 action on target cells are believed to be mediated through activation of kinase cascades that have also been associated with cellular stresses, such as the stress activated MAP kinases JNK/SAPK and p38.

[0004] A third member of the IL-1 family was subsequently discovered which acts as a natural antagonist of IL-1 α and IL-1 β by binding to the IL-1 receptor but not transducing an intracellular signal or a biological response. The protein was called IL-1ra (for IL-1 receptor antagonist) or IRAP (for IL-1 receptor antagonist protein). At least three alternatively splice forms of IL-1ra exist: one encodes a secreted protein, and the other two encode intracellular proteins. The relative role of the three forms and reason for their different localization is not known. All three proteins, IL-1 α , IL-1 β and IL-1ra share approximately 25-30% amino acid identity and a similar three-dimensional structure consisting of twelve β -strands folded into a β -barrel, with an internal thrice repeated structural motif.

[0005] There are three known IL-1 receptor subunits. The active receptor complex consists of the type I receptor and IL1RAcP (for IL-1 accessory protein). The type I receptor is responsible for binding of the three ligands, and is able to do so in the absence of the IL1RAcP. However signal transduction requires interaction of IL-1 α or β with the IL1RAcP. IL-1ra does not interact with the IL-1RAcP and hence cannot signal. A third receptor subunit, the type II receptor, binds IL-1 α and IL-1 β but cannot signal due to its lack of an intracellular domain. Rather it act as a decoy either in its membrane form or an antagonist in a cleaved secreted form, and hence inhibits IL-1 activity. It only weakly binds IL-1ra.

[0006] Many studies using IL-1ra, soluble IL-1R, derived from the extracellular domain of the type I IL-1R, antibodies to IL-1 α or β , and transgenic knockouts of these genes have shown conclusively that the IL-1s play a key role in a number of pathophysiologies (see C.A. Dinarello, Blood 87:2095-2147 (1996) for a review). For example, IL-1ra has been shown to be effective in animal models of septic shock, rheumatoid arthritis, graft versus host disease, stroke, cardiac ischemia, and is currently in clinical trials for some of these indications. Moreover, IL-1 α and β have shown some potential as hematopoietic stem cell stimulators with potential as radio- and chemoprotectants.

[0007] More recently, a more distant member of the IL-1 family was identified. This protein, originally isolated through its ability to induce Interferon gamma in T cells and hence called Interferon gamma inducing factor (IGIF) [H. Okamura et al., Nature 378:88-91 (1995)], was subsequently shown to fold in a similar structure to the IL-1s and share weak amino acid identity [Bazan et al., Nature 379:591 (1996)]. The name IL-1 γ was proposed. IGIF appears to play a direct role in the liver damage which occurs during toxic shock and is therefore like the other IL-1s in playing an early role in inflammatory and stressful conditions. This indicates that these Interleukin-1s have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of Interleukin-1 family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, chronic and acute inflammation, septicemia, shock, arthritis, inflammatory bowel disease, graft vs. host disease, autoimmunity, stroke, cardiac ischemia, acute respiratory disease syndrome (ARDS), psoriasis, restenosis, traumatic brain injury, AIDS, cancer, anemia, and cachexia. WO96/09323 is concerned with the production and use of mutants of the IL-1 receptor antagonist IL-1ra, which mutants have increased affinity for the IL-1 receptor, in particular for the type 1 receptor. Such mutants may be effectively used in the manufacture of drugs for inhibiting IL-1 activity.

[0008] In one aspect, the invention relates to IL-1ra beta polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such IL-1ra beta polypeptides and poly-

nucleotides in the manufacture of a medicament for the treatment of a subject in need of enhanced IL-1ra beta polypeptide activity. Such uses include the treatment of chronic and acute inflammation, septicemia, cancer, anemia, arthritis, inflammatory bowel disease, graft vs. host disease, autoimmunity, stroke, shock, cardiac ischemia, acute respiratory disease syndrome (ARDS), psoriasis, restenosis, traumatic brain injury, AIDS, cancer, anemia, cachexia, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with IL-1ra beta imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate IL-1ra beta activity or levels.

[0009] Figure 1 shows the nucleotide and deduced amino acid sequence of human IL-1ra beta. SEQ ID NOS: 1 and 2.

[0010] The following definitions are provided to facilitate understanding of certain terms used frequently herein.

[0011] "IL-1ra beta" refers generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

[0012] "IL-1ra beta activity or IL-1ra beta polypeptide activity" or "biological activity of the IL-1ra beta or IL-1ra beta polypeptide" refers to the metabolic or physiologic function of said IL-1ra beta including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said IL-1ra beta.

[0013] "IL-1ra beta polypeptides" refers to polypeptides with amino acid sequences sufficiently similar to IL-1ra beta sequences, preferably exhibiting at least one biological activity of the IL-1ra beta.

[0014] "IL-1ra beta gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO: or allelic variants thereof and/or their complements.

[0015] "IL-1ra beta polynucleotides" refers to polynucleotides containing a nucleotide sequence which encodes a IL-1ra beta polypeptide or fragment thereof, or a nucleotide sequence which has at least 80% identity to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or the corresponding fragment thereof, or a nucleotide sequence which has sufficient 80% identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker.

[0016] "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

[0017] "Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

[0018] "Polynucleotide" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

[0019] "Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phos-

photidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

[0020] "Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

[0021] "Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. *et al.*, *J. Molec. Biol.* 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894; Altschul, S., *et al.*, *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

[0022] Preferred parameters for polypeptide sequence comparison include the following:

- 1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)
- Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992)
- Gap Penalty: 12
- Gap Length Penalty: 4

[0023] A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps).

[0024] Preferred parameters for polynucleotide comparison include the following:

- 1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)
- Comparison matrix: matches = +10, mismatch = 0
- Gap Penalty: 50
- Gap Length Penalty: 3

[0025] A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polynucleotide comparisons.

[0026] Preferred polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polynucleotide reference sequence of SEQ ID NO:

5 1, wherein said reference sequence may be identical to the sequence of SEQ ID NO: 1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the numerical percent of the respective percent identity and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

10

$$n_n \leq x_n - (x_n \cdot y)$$

15

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, and y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

[0027] Preferred polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said reference sequence may be identical to the sequence of SEQ ID NO: 2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity and subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y),$$

35

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, and y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

Polypeptides of the Invention

[0028] The IL-1ra beta polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as IL-1ra beta polypeptides and which have at least 80% identity to the polypeptide of SEQ ID NO:2 or the relevant portion and more preferably at least 85% identity, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2.

[0029] The IL-1ra beta polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

[0030] Biologically active fragments of the IL-1ra beta polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned IL-1ra beta polypeptides. As with IL-1ra beta polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of IL-1ra beta polypeptide. In this context

"about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

[0031] Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of IL-1ra beta polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Biologically active fragments are those that mediate IL-1ra beta activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

[0032] Thus, the polypeptides of the invention include polypeptides having an amino acid sequence at least identical to that of SEQ ID NO:2 or fragments thereof with at least 80% identity to the corresponding fragment of SEQ ID NO:2. Preferably, all of these polypeptides retain the biological activity of the IL-1ra beta, including antigenic activity. Included in this group are variants of the defined sequence and fragments. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

[0033] The IL-1ra beta polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

[0034] Another aspect of the invention relates to isolated polynucleotides which encode the IL-1ra beta polypeptides and polynucleotides closely related thereto.

[0035] IL-1ra beta of the invention is structurally related to other proteins of the Interleukin-1 family, as shown by the results of sequencing the cDNA encoding human IL-1ra beta. The cDNA sequence contains an open reading frame encoding a protein of 169 amino acids with a deduced molecular weight of 18.7 kDa. IL-1ra beta of Figure 1 (SEQ ID NO:2) has about 29.9% identity (using BESTFIT (part of GCG suite of programs)) in amino acid residues with human IL-1 receptor antagonist (IL-1ra) (S.P. Eisenberg et al., *Nature* 343:341-346, 1990) over 162 residues. Furthermore, IL-1ra beta (SEQ ID NO:2) is 21.3% identical to human Interleukin 1 beta (IL-1beta) over 160 residues (P.E. Auron et al., *Proc Natl. Acad. Sci. USA* 81:7907-7911, 1984; C.J. March et al., *Nature* 315:641-647 (1985)). IL-1ra beta gene of Figure 1 (SEQ ID NO:1) has about 59.0 % identity (using BESTFIT (part of the GCG suite of programs)) in 230 nucleotide residues with human IL-1ra [S.P. Eisenberg et al., *Nature* 343:341-346, 1990].

[0036] One polynucleotide of the present invention encoding IL-1ra beta may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human keratinocytes and TNF α plus IFN γ (Interferon γ) induced epithelial cells using the expressed sequence tag (EST) analysis (Adams, M.D., et al. *Science* (1991) 252: 1651-1656; Adams, M.D. et al., *Nature*, (1992) 355:632-634; Adams, M.D., et al., *Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

[0037] Thus, the nucleotide sequence encoding IL-1ra beta polypeptides may be identical over its entire length to the coding sequence in Figure 1 (SEQ ID NO:1), or may be a degenerate form of this nucleotide sequence encoding the polypeptide of SEQ ID NO:2, or may be highly identical to a nucleotide sequence that encodes the polypeptide of SEQ ID NO:2. Preferably, the polynucleotides of the invention contain a nucleotide sequence that is highly identical, at least identical, with a nucleotide sequence encoding a IL-1ra beta polypeptide, or at least 80% identical with the encoding nucleotide sequence set forth in Figure 1 (SEQ ID NO: 1), or at least 80% identical to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2.

[0038] When the polynucleotides of the invention are used for the recombinant production of IL-1ra beta polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided

in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

[0039] Among particularly preferred embodiments of the invention are polynucleotides encoding IL-1ra beta polypeptides having the amino acid sequence of set out in Figure 1 (SEQ ID NO:2) and variants thereof.

[0040] Further preferred embodiments are polynucleotides encoding IL-1ra beta variants that have the amino acid sequence of the IL-1ra beta polypeptide of Figure 1 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

[0041] Further preferred embodiments of the invention are polynucleotides that are at least 80% identical over their entire length to a polynucleotide encoding the IL-1ra beta polypeptide having the amino acid sequence set out in Figure 1 (SEQ ID NO:2), and polynucleotides which are complementary to such polynucleotides. In this regard, polynucleotides at least 80% identical over their entire length to the same are particularly preferred, and those with at least 90% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred.

[0042] The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

[0043] Polynucleotides of the invention, which are sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding IL-1ra beta polypeptide and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the IL-1ra beta gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 70% identical, preferably 80% identical, more preferably 90% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

[0044] The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

30 Vectors, Host Cells, Expression

[0045] The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

[0046] For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis *et al.*, *BASIC METHODS IN MOLECULAR BIOLOGY*(1986) and Sambrook *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

[0047] Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

[0048] A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL (supra)*.

[0049] For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide.

These signals may be endogenous to the polypeptide or they may be heterologous signals.

[0050] If the IL-1ra beta polypeptide is to be expressed for use in screening assays, the polypeptide may be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If IL-1ra beta polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered. IL-1ra beta polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

[0051] This invention also relates to the use of IL-1ra beta polynucleotides for use as diagnostic reagents. Detection of a mutated form of IL-1ra beta gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of IL-1ra beta. Individuals carrying mutations in the IL-1ra beta gene may be detected at the DNA level by a variety of techniques.

[0052] Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled IL-1ra beta nucleotide sequences.

Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cottrell *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising fragments from IL-1ra beta nucleotide sequences can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

[0053] The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, septicemia, cancer, anemia, arthritis, inflammatory bowel disease, graft vs. host disease, autoimmunity, stroke, cardiac ischemia, acute respiratory disease syndrome (ARDS), cancer, anemia, psoriasis, restenosis, traumatic brain injury, AIDS, cachexia. through detection of mutation in the IL-1ra beta gene by the methods described.

[0054] In addition, chronic and acute inflammation, septicemia, cancer, anemia, arthritis, inflammatory bowel disease, graft vs. host disease, autoimmunity, stroke, cardiac ischemia, acute respiratory disease syndrome (ARDS), psoriasis, restenosis, traumatic brain injury, AIDS, cancer, anemia, and cachexia can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of IL-1ra beta polypeptide or IL-1ra beta mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an IL-1ra beta polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Chromosome Assays

[0055] The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data.

Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man* (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

[0056] The IL-1ra beta gene was mapped to chromosome 2, in a region close to IL-1 α , β and IL-1ra by comparing with public databases containing sequences obtained from mapped fragments of genomic DNA obtained by PCR using synthetic oligonucleotide primer pairs.

[0057] The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

[0058] The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the IL-1ra beta polypeptides. The term "immunospecific" means that the antibodies have substantial greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

[0059] Antibodies generated against the IL-1ra beta polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983)4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

[0060] Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

[0061] The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

[0062] Antibodies against IL-1ra beta polypeptides may also be employed to treat chronic and acute inflammation, septicemia, cancer, anemia, arthritis, inflammatory bowel disease, graft vs. host disease, autoimmunity, stroke, cardiac ischemia, acute respiratory disease syndrome (ARDS), psoriasis, restenosis, traumatic brain injury, AIDS, cancer, anemia, cachexia, among others.

Vaccines

[0063] Another aspect of the invention relates to a method for the manufacture of a medicament for inducing an immunological response in a mammal which comprises inoculating the mammal with IL-1ra beta polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, septicemia, cancer, anemia, arthritis, inflammatory bowel disease, graft vs. host disease, autoimmunity, stroke, cardiac ischemia, acute respiratory disease syndrome (ARDS), psoriasis, restenosis, traumatic brain injury, AIDS, cancer, anemia, cachexia, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering IL-1ra beta gene via a vector directing expression of IL-1ra beta polypeptide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

[0064] Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a IL-1ra beta polypeptide wherein the composition comprises a IL-1ra beta polypeptide or IL-1ra beta gene. The vaccine formulation may further comprise a suitable carrier. Since IL-1ra beta polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

55

Screening Assays

[0065] The IL-1ra beta polypeptide of the present invention may be employed in a screening process for compounds

which stimulate (agonists) or inhibit (antagonists, or otherwise called inhibitors) the synthesis or action of the IL-1ra beta polypeptide of the present invention. The IL-1ra beta polypeptide of the present invention may also be employed in a screening process for compounds which mimic the agonist or antagonist properties of the IL-1ra beta polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess and identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural substrates, ligands, receptors, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

[0066] IL-1ra beta proteins are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate IL-1ra beta polypeptide on the one hand and which can inhibit the function of IL-1ra beta polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, septicemia, arthritis, inflammatory bowel disease, graft vs. host disease, autoimmunity, stroke, cardiac ischemia, acute respiratory disease syndrome (ARDS), psoriasis, restenosis, traumatic brain injury, AIDS, cachexia.. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, septicemia, cancer, anemia, arthritis, inflammatory bowel disease, graft vs. host disease, autoimmunity, stroke, shock, atherosclerosis, cardiac ischemia, acute respiratory disease syndrome (ARDS), psoriasis, restenosis, traumatic brain injury, AIDS, cancer, anemia, cachexia..

[0067] In general, such screening procedures may involve identifying, generating and using appropriate cells which express the receptor of the IL-1ra beta polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Such cells may be identified, for example, by direct binding methods using radiolabeled or fluorescently tagged IL-1ra beta polypeptide. Cells expressing the IL-1ra beta polypeptide receptor (or cell membrane containing the expressed polypeptide) are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. Alternatively, the cDNA for the IL-1ra beta polypeptide receptor may be cloned by the above direct binding methods using expression cloning or purification methods known in the art, and its extracellular domain expressed as a secreted or membrane protein. The soluble or membrane bound receptor can then be used to identify agonists or antagonists via direct binding methods.

[0068] The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the IL-1ra beta polypeptide receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled IL-1ra beta polypeptide. Further, these assays may test whether the candidate compound results in a signal similar to that generated by binding of the IL-1ra beta polypeptide, using detection systems appropriate to the cells bearing the IL-1ra beta polypeptide receptor at their surfaces. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for conducting such screening assays are well understood in the art.

[0069] Examples of potential IL-1ra beta polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, etc., as the case may be, of the IL-1ra beta polypeptide, e.g., a fragment of the ligands, substrates, receptors, or small molecules which bind to the target receptor of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

40 Prophylactic and Therapeutic Methods

[0070] This invention provides methods for the manufacture of a medicament for treating an abnormal conditions related to both an excess of and insufficient amounts of IL-1ra beta polypeptide activity.

[0071] If the activity of IL-1ra beta polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as herein above described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of the IL-1ra beta polypeptide to its target receptor, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

[0072] In another approach, soluble forms of IL-1ra beta polypeptides capable of binding its receptor in competition with endogenous IL-1ra beta polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the IL-1ra beta polypeptide.

[0073] In still another approach, expression of the gene encoding endogenous IL-1ra beta polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

[0074] For treating abnormal conditions related to an under-expression of IL-1ra beta and its activity, several approaches are also available. Alternatively, gene therapy may be employed to effect the endogenous production of IL-1ra beta by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

10 Formulation and Administration

[0075] Peptides, such as the soluble form of IL-1ra beta polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

[0076] Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

[0077] Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

[0078] The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

[0079] Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

40 Examples

[0080] The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

45 Example 1

Isolation and identification of IL-1RAβ

[0081] A potential full length clone (HGS EST #1506331; Project ID HAICQ62) was initially identified through a search of the Human Genome Sciences EST database (*vide supra* for EST analysis) for proteins with homology to members of the interleukin 1 family. This partial sequence showed significant sequence identity (35% over 77 aa) to murine IL-1ra. This cDNA was completely sequenced on both strands using an automated sequencer. A total of 1183 bp were sequenced, and this includes an open reading frame encoding a peptide of 169 aa. The cDNA and protein sequences are SEQ ID NOS: 1 and 2, respectively, and are named IL-1raβ. The protein does not appear to have a signal sequence at its amino terminus and is likely to be expressed as an intracellular, cytosolic protein like other members of the family. It is possible that alternative splice forms exist which include a signal sequence, as has been found for IL-1ra.

[0082] The IL-1ra beta gene was mapped to chromosome 2, in a region close to IL-1α, β and IL-1ra by comparing with public databases containing sequences obtained from mapped fragments of genomic DNA obtained by PCR using

synthetic oligonucleotide primer pairs. Using the algorithm BLAST, a match was found with human STS CHLC. GAAT11C03.P3330 clone GAAT11C03 (Accession number G942011) which can be mapped to chromosome 2 approximately 142 cM from the top of the chromosome.

5 SEQUENCE LISTING

[0083]

10 (1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: Interleukin-1 Receptor Antagonist Beta (IL-1RA β)

15 (iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

20 (A) ADDRESSEE: F J Cleveland & Company

(B) STREET: 40/43 Chancery Lane

(C) CITY: London

(D) COUNTY:

(E) COUNTRY: United Kingdom

(F) POST CODE: WC2A 1JQ

25 (v) COMPUTER READABLE FORM:

30 (A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

(D) SOFTWARE: Fast SEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

35 (A) APPLICATION NUMBER:

(B) FILING DATE: 28-JAN-1997

(C) CLASSIFICATION:

40 (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

45 (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: CRUMP, Julian Richard John

(B) GENERAL AUTHORISATION NUMBER: 37127

(C) REFERENCE/DOCKET NUMBER: ATG50051

50 (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +44 171 405 5875

(B) TELEFAX: +44 171 831 0749

(C) TELEX:

55 (2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1183 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

10

GGCAGGAGCC	ACGATT CAGT	CCCCGGACT	GTAGAT AAAG	ACCCCTT CTT	GCCAGGT GCT	60	
GAGACAACCA	CACT ATGAGA	GGCACT CCAG	GAGACG CTGA	TGGTGGAGGA	AGGGCCGT CT	120	
15	AT CAAT CAAT	GTGTAAACCT	ATTACTGGGA	CTATT AATGA	TTTGAAT CAG	CAAGTGTGGA	180
CCCTT CAGGG	T CAGAACCTT	GTGGCAGTTC	CACGAAGTGA	CAGTGTGACC	CCAGT CACTG	240	
TTGCTTAT	CACATGCAAG	TATCCAGAGG	CTCTTGAGCA	AGGCAGAGGG	GATCCCATT	300	
ATTTGGGAAT	CCAGAATCCA	GAAATGTGTT	TGTATTGTGA	GAAGGTTGGA	GAACAGCCCA	360	
20	CATTGAGCT	AAAAGAGCAG	AAGATCATGG	ATCTGTATGG	CCAACCCGAG	CCCGTGAAAC	420
CCTT CTTTT	CTACCGTGCC	AAGACTGGTA	GGACCTCCAC	CCTTGAGT CT	GTGGCCTTCC	480	
CGGACTGGTT	CATTGCCTCC	TCCAAGAGAG	ACCAGCCCAT	CATTCTGACT	T CAGAACTTG	540	
GGAAGTCATA	CAACACTGCC	TTTGAATTAA	ATATAAATGA	CTGAACTCAG	CCTAGAGGTG	600	
25	GCAGCTTGGT	CTTTGTCTTA	AAGTTTCTGG	TTCCCAATGT	GTTTCTGCT	ACATTTTCTT	660
AGTGT CATT	T CACGCTGGT	GCTGAGACAG	GGGCAAGGCT	GCTGTTATCA	TCTCATTAA	720	
TAATGAAGAA	GAAGCAATT	CTT CATAAGCA	ACTGAAGAAC	AGGATGTGGC	CTCAGAAGCA	780	
GGAGAGCTGG	GTGGTATAAG	GCTGT CCTCT	CAAGCTGGTC	CTGTGTAGGC	CACAAGGCAT	840	
30	CTGCATGAGT	GACTTTAAGA	CTCAAAGACC	AAACACTGAG	CTTTCTTCTA	GGGGTGGGTA	900
TGAAGATGCT	T CAGAGCTCA	TGCGCGTTAC	CCACGATGGC	ATGACTAGCA	CAGAGCTGAT	960	
CTCTGTTCT	GTGGTCTTT	ATTCCCTTT	GGGATGATAT	CATCCAGTCT	TTATATGTTG	1020	
35	CCAATATACC	T CATTGTGTG	TAATAGAACCT	TTCTTAGCAT	TAAGACCTTG	TAAACAAAAAA	1080
TAATTCTTGT	GTAAAGTTAA	ATCATTGTTG	TCCATTGT	AATGTGTAAAT	CTTAAAGTTA	1140	
AATAAACTTT	GTGTATTTAT	ATAATAAAAAA	AAAAAAAAAAA	AAA		1183	

40

45

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

50

(A) LENGTH: 169 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Arg Gly Thr Pro Gly Asp Ala Asp Gly Gly Gly Arg Ala Val Tyr
 1 5 10 15
 5 Gln Ser Met Cys Lys Pro Ile Thr Gly Thr Ile Asn Asp Leu Asn Gln
 20 25 30
 Gln Val Trp Thr Leu Gln Gly Gln Asn Leu Val Ala Val Pro Arg Ser
 35 40 45
 10 Asp Ser Val Thr Pro Val Thr Val Ala Val Ile Thr Cys Lys Tyr Pro
 50 55 60
 Glu Ala Leu Glu Gln Gly Arg Gly Asp Pro Ile Tyr Leu Gly Ile Gln
 65 70 75 80
 15 Asn Pro Glu Met Cys Leu Tyr Cys Glu Lys Val Gly Glu Gln Pro Thr
 85 90 95
 Leu Gln Leu Lys Glu Gln Lys Ile Met Asp Leu Tyr Gly Gln Pro Glu
 100 105 110
 20 Pro Val Lys Pro Phe Leu Phe Tyr Arg Ala Lys Thr Gly Arg Thr Ser
 115 120 125
 Thr Leu Glu Ser Val Ala Phe Pro Asp Trp Phe Ile Ala Ser Ser Lys
 130 135 140
 25 Arg Asp Gln Pro Ile Ile Leu Thr Ser Glu Leu Gly Lys Ser Tyr Asn
 145 150 155 160
 Thr Ala Phe Glu Leu Asn Ile Asn Asp
 30 165

Claims

35 1. An isolated Interleukin-1 polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the IL-1ra beta polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence.

40 2. The polynucleotide of claim 1 which is DNA or RNA.

3. The polynucleotide of claim 1 wherein said nucleotide sequence is at least 80% identical to that contained in SEQ ID NO:1.

45 4. The polynucleotide of claim 3 wherein said nucleotide sequence comprises the IL-1ra beta polypeptide encoding sequence contained in SEQ ID NO:1.

5. The polynucleotide of SEQ ID NO: 1.

50 6. A DNA or RNA molecule comprising an expression system, which expression system is capable of producing an IL-1ra beta polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the DNA or RNA molecule of claim 6.

55 8. An ex vivo process for producing a IL-1ra beta polypeptide comprising culturing the host of claim 7 under conditions sufficient for the production of said polypeptide.

9. The process of claim 8 which further includes recovering said polypeptide.
10. A process for producing a cell which produces an IL-1ra beta polypeptide, comprising transforming or transfecting a host cell with the DNA or RNA molecule of claim 6 such that the host cell, under appropriate culture conditions, produces an IL-1ra beta polypeptide.
5
11. Cells produced by the process of claim 10.
12. An IL-1ra beta polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.
10
13. The polypeptide of claim 12 which comprises the amino acid sequence of SEQ ID NO:2.
14. The polypeptide of SEQ ID NO: 2
15
15. A IL-1ra beta polypeptide prepared by the process of claim 8.
16. An antibody immunospecific for the IL-1ra beta polypeptide of claim 12.
20
17. Use of the IL-1ra beta polypeptide of any of claims 12 - 15 in the manufacture of a medicament for the treatment of a subject in need of enhanced IL-1ra beta polypeptide activity, which medicament is capable of stimulating IL-1ra beta polypeptide activity in vivo.
18. Use of the IL-1ra polypeptide of any of claims 12-15 or a biologically active fragment of said polypeptide, in the manufacture of a medicament for the treatment of a subject in need of reduced IL-1ra beta polypeptide activity.
25
19. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of IL-1ra beta polypeptide in said subject, comprising:
30
 - (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said IL-1ra beta polypeptide in the genome of said subject; and/or
 - (b) analyzing for the presence or amount of the IL-1ra beta polypeptide expression in a sample derived from said subject.
20. A method for identifying compounds which inhibit (antagonize) or agonize the IL-1ra beta polypeptide of any of claims 12 to 15, which comprises:
35
 - (a) contacting a candidate compound with cells or cell membranes which express said IL-1ra beta polypeptide or respond to said IL-1ra beta polypeptide; and
 - (b) observing the binding, or stimulation or inhibition of a functional response; or comparing the ability of the cells or cell membranes which were contacted with the candidate compounds with similar cells or cell membranes which were not contacted for IL-1ra beta polypeptide activity.
40
21. A polypeptide obtainable by expressing a nucleotide sequence comprising the sequence of SEQ ID NO:1.
45

Patentansprüche

1. Isoliertes Interleukin-1 Polynukleotid, das eine Nukleotidsequenz umfaßt, die zu mindestens 80 % mit einer Nukleotidsequenz identisch ist, die das IL-1ra beta Polypeptid der SEQ ID NR: 2 über die gesamte Länge codiert, oder eine Nukleotidsequenz, die komplementär zur besagten Nukleotidsequenz ist.
50
2. Polynukleotid nach Anspruch 1, welches DNA oder RNA ist.
3. Polynukleotid nach Anspruch 1, wobei die Nukleotidsequenz zu mindestens 80 % identisch zu der in SEQ ID NR: 1 enthaltenen Nukleotidsequenz ist.
55
4. Polynukleotid nach Anspruch 3, wobei die Nukleotidsequenz die IL-1ra beta Polypeptid-codierende Sequenz, ent-

halten in SEQ ID NR: 1, umfaßt.

5. Polynukleotid mit der SEQ ID NR:1.

5 6. DNA- oder RNA-Molekül, das ein Expressionssystem umfaßt, das in der Lage ist, ein IL-1ra beta Polypeptid zu produzieren, das eine Aminosäuresequenz umfaßt, die zu mindestens 80 % identisch mit dem Polypeptid der SEQ ID NR:2 ist, wenn das Expressionssystem in einer kompatiblen Wirtszelle vorliegt.

10 7. Wirtszelle, die das DNA- oder RNA-Molekül nach Anspruch 6 umfaßt.

8. *Ex vivo*-Prozess zur Herstellung eines IL-1ra beta Polypeptids, das die Züchtung des Wirts nach Anspruch 7 unter Bedingungen umfaßt, die zur Herstellung des Polypeptids ausreichend sind.

15 9. Verfahren nach Anspruch 8, welches ferner die Gewinnung des Polypeptids einschließt.

10 10. Verfahren zur Herstellung einer Zelle, die ein IL-1ra beta Polypeptid produziert, umfassend die Transformation oder Transfektion einer Wirtszelle mit dem DNA- oder RNA-Molekül nach Anspruch 6, so daß die Wirtszelle unter geeigneten Kultivierungsbedingungen ein IL-1ra beta Polypeptid produziert.

20 11. Zellen, hergestellt durch das Verfahren nach Anspruch 10.

12. IL-1ra beta Polypeptid, das eine Aminosäuresequenz umfaßt, die zu mindestens 80 % mit der Aminosäuresequenz der SEQ ID NR:2 über die gesamte Länge identisch ist.

25 13. Polypeptid nach Anspruch 12, das die Aminosäuresequenz der SEQ ID NR:2 umfaßt.

14. Polypeptid mit der SEQ ID NR:2.

15. IL-1ra beta Polypeptid, hergestellt durch das Verfahren nach Anspruch 8.

30 16. Antikörper, der immunspezifisch für das IL-1ra beta Polypeptid nach Anspruch 12 ist.

35 17. Verwendung des IL-1ra beta Polypeptids nach einem der Ansprüche 12 bis 15 zur Herstellung eines Medikaments zur Behandlung eines Patienten, der eine verstärkte IL-1ra beta Polypeptid-Aktivität benötigt, wobei das Medikament in der Lage ist, die IL-1ra beta Polypeptid-Aktivität *in vivo* zu stimulieren.

40 18. Verwendung des IL-1ra Polypeptids nach einem der Ansprüche 12 bis 15 oder eines biologisch aktiven Fragments des Polypeptids zur Herstellung eines Medikaments zur Behandlung eines Patienten, der eine reduzierte IL-1ra beta Polypeptid-Aktivität benötigt.

45 19. Verfahren zur Diagnose einer Krankheit oder Anfälligkeit für eine Krankheit in einem Subjekt, die mit der Expression oder Aktivität des IL-1ra beta Polypeptids in dem Subjekt verbunden ist, umfassend:

(a) Bestimmen des Auftretens oder Fehlens einer Mutation in der Nukleotidsequenz, die das IL-1ra beta Polypeptid codiert, in dem Genom des Subjekts; und/oder

(b) Analyse auf das Vorliegen oder die Höhe der IL-1ra beta Polypeptid-Expression in einer Probe, die von dem Subjekt stammt.

50 20. Verfahren zur Identifizierung von Verbindungen, die das IL-1ra beta Polypeptid nach einem der Ansprüche 12 bis 15 inhibieren (als Antagonist fungieren) oder als Agonist fungieren, umfassend:

(a) In Kontakt bringen einer zu analysierenden Verbindung mit Zellen oder Zellmembranen, die das IL-1ra beta Polypeptid exprimieren oder auf das IL-1ra beta Polypeptid reagieren; und

(b) Feststellung der Bindung oder Stimulation oder Inhibition einer funktionalen Antwort; oder Vergleich der Fähigkeit der Zellen oder Zellmembranen, die mit der zu analysierenden Verbindung in Kontakt gebracht wurden mit ähnlichen Zellen oder Zellmembranen, die nicht in Kontakt gebracht wurden auf IL-1ra beta Polypeptid-Aktivität hin.

21. Polypeptid, das durch Expression einer Nucleotidsequenz, die die Sequenz der SEQ ID NR:1 umfaßt, erhältlich ist.

Revendications

5 1. Polynucléotide d'interleukine-1 isolé, comprenant une séquence de nucléotides qui présente une identité d'au moins 80 % avec une séquence de nucléotides codant pour le polypeptide IL-1ra bêta de la SEQ ID N° 2 sur sa longueur totale ; ou une séquence de nucléotides complémentaire de ladite séquence de nucléotides.

10 2. Polynucléotide suivant la revendication 1, qui consiste en ADN ou ARN.

15 3. , Polynucléotide suivant la revendication 1, dans lequel la séquence de nucléotides est identique à au moins 80 % à celle présente dans la SEQ ID N° 1.

20 4. Polynucléotide suivant la revendication 3, dans lequel la séquence de nucléotides comprend la séquence codant pour le polypeptide IL-1ra bêta présente dans la SEQ N° 1.

25 5. Polynucléotide de la SEQ ID N° 1.

30 6. Molécule d'ADN ou d'ARN comprenant un système d'expression, système d'expression qui est capable de produire un polypeptide IL-1ra bêta comprenant une séquence d'amino-acides qui présente une identité d'au moins 80 % avec le polypeptide de la SEQ ID N° 2 lorsque ledit système d'expression est présent dans une cellule hôte compatible.

35 7. Cellule hôte comprenant la molécule d'ADN ou d'ARN suivant la revendication 6.

40 8. Procédé ex vivo pour la production d'un polypeptide IL-1ra bêta, comprenant la culture de l'hôte suivant la revendication 7 dans des conditions suffisantes pour la production dudit polypeptide.

45 9. Procédé suivant la revendication 8, qui comprend en outre l'étape consistant à recueillir le polypeptide.

50 10. Procédé pour la production d'une cellule qui produit un polypeptide IL-1ra bêta, comprenant la transformation ou la transfection d'une cellule hôte avec la molécule d'ADN ou d'ARN suivant la revendication 6 de telle sorte que la cellule hôte, dans des conditions de culture appropriées, produise un polypeptide IL-1ra bêta.

55 11. Cellules produites par le procédé suivant la revendication 10.

60 12. Polypeptide IL-1ra bêta comprenant une séquence d'amino-acides qui est identique à au moins 80 % à la séquence d'amino-acides de la SEQ ID N° 2 sur sa longueur totale.

65 13. Polypeptide suivant la revendication 12, qui comprend la séquence d'amino-acides de la SEQ ID N° 2.

70 14. , Polypeptide de la SEQ ID N° 2.

75 15. Polypeptide IL-1ra bêta préparé par le procédé suivant la revendication 8.

80 16. Anticorps immunospécifique pour le polypeptide IL-1ra bêta suivant la revendication 12.

85 17. Utilisation du polypeptide IL-1ra bêta suivant l'une quelconque des revendications 12 à 15 dans la production d'un médicament destiné au traitement d'un sujet nécessitant une activité accrue du polypeptide IL-1ra bêta, médicament qui est capable de stimuler l'activité du polypeptide IL-1ra bêta in vivo.

90 18. Utilisation du polypeptide IL-1ra suivant l'une quelconque des revendications 12 à 15 ou d'un fragment biologiquement actif de ce polypeptide, dans la production d'un médicament destiné au traitement d'un sujet nécessitant une activité réduite du polypeptide IL-1ra bêta.

95 19. Procédé pour diagnostiquer une maladie ou une prédisposition à une maladie chez un sujet en rapport avec l'expression ou l'activité du polypeptide IL-1ra bêta chez ledit sujet, comprenant les étapes consistant :

(a) à déterminer la présence ou l'absence d'une mutation dans la séquence de nucléotides codant pour ledit polypeptide IL-1ra bêta dans le génome dudit sujet ; et/ou
(b) à effectuer une analyse pour déterminer la présence ou la quantité exprimée du polypeptide IL-1ra bêta dans un échantillon provenant dudit sujet.

5 20. Méthode d'identification de composés qui inhibent (antagonisent) ou agonisent le polypeptide IL-1ra bêta suivant l'une quelconque des revendications 12 à 15, qui comprend les étapes consistant :

10 (a) à mettre en contact un composé candidat avec des cellules ou membranes cellulaires qui expriment ledit polypeptide IL-1ra bêta ou qui répondent audit polypeptide IL-1ra bêta ; et
(b) à observer la liaison, ou la stimulation ou bien l'inhibition d'une réponse fonctionnelle ; ou à comparer l'aptitude des cellules ou membranes cellulaires qui ont été mises en contact avec les composés candidats à des cellules ou membranes cellulaires similaires qui n'ont pas été mises en contact, en ce qui concerne l'activité du polypeptide IL-1ra bêta.

15 21. Polypeptide pouvant être obtenu par expression d'une séquence de nucléotides comprenant la séquence de la SEQ ID N° 1.

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FIGURE 1. Nucleotide and Amino Acid sequence of IL-1ra beta (SEQ ID NQS: 1 and 2, respectively.)

SEQ ID NO. 1

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1   GGCACGAGCC ACGATT CAGT CCCCTGGACT GTAGATAAAAG ACCCTTT CTT
51  GCCAGGTGCT GAGACAACCA CACTATGAGA GGCACCTCCAG GAGACGCTGA
101 TGGTGGAGGA AGGGCCGTCT ATCAATCAAT GTGTAAACCT ATTACTGGGA
151 CTATTAATGA TTTGAATCAG CAAGTGTGGA CCCTTCAGGG TCAGAACCTT
201 GTGGCAGTTC CACGAAGTGA CAGTGTGACC CCAGTCAGT TTGCTGTTAT
251 CACATGCAAG TATCCAGAGG CTCTTGAGCA AGGCAGAGGG GATCCCATTT
301 ATTTGGGAAT CCAGAATCCA GAAATGTGTT TGTATTGTGA GAAGGTTGGA
351 GAACAGCCCA CATTGCAGCT AAAAGAGCAG AAGATCATGG ATCTGTATGG
401 CCAACCCGAG CCCGTGAAAC CCTTCCTTT CTACCGTGCC AAGACTGGTA
451 GGACCTCCAC CCTTGAGTCT GTGGCCTTCC CGGACTGGTT CATTGCCTCC
501 TCCAAGAGAG ACCAGCCCCT CATTCTGACT TCAGAACTTG GGAAGTCATA
551 CAACACTGCC TTTGAATTAA ATATAATGA CTGAACTCAG CCTAGAGGTG
601 GCAGCTTGGT CTTTGTCTTA AAGTTTCTGG TTCCCAATGT GTTTTCTGCT
651 ACATTTCTT AGTGTCTTT TCACGCTGGT GCTGAGACAG GGGCAAGGCT
701 GCTGTTATCA TCTCATTAA TAATGAAGAA GAAGCAATTA CTTCATAGCA
751 ACTGAAGAAC AGGATGTGGC CT CAGAAGCA GGAGAGCTGG GTGGTATAAG
801 GCTGTCTCT CAAGCTGGT CTGTGTAGGC CACAAGGCAT CTGCATGAGT
851 GACTTTAAGA CTCAAAGACC AAACACTGAG CTTCTTCTA GGGGTGGGTA
901 TGAAGATGCT T CAGAGCTCA TGCGCGTTAC CCACGATGGC ATGACTAGCA
951 CAGAGCTGAT CTCTGTTCT GTTTTGTCTT ATTCCCTCTT GGGATGATAT
1001 CATCCAGTCT TTATATGTTG CCAATATACC TCATTGTGTG TAATAGAAC
1051 TTCTTAGCAT TAAGACCTTG TAAACAAAAA TAATTCTTGT GTTAAGTTAA
1101 ATCATTTTTG TCCTAATTGT AATGTGTAAT CTTAAAGTTA AATAAACTTT
1151 GTGTATTTAT ATAATAAAAAA AAAAAAAA AAA

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SEQ ID NO. 2

1 MRGTPGDADG GGRAYYQSMC KPITGTINDL NQQVWTLQGQ NLVAVP RSD S
51 VTPVTVAVIT CKYPEALEQG RGDPIYLGQ NPEMCLYCEK VGEQPTLQLK
101 EQKIMDLYGQ PEPVKPFLFY RAKTGRSTL ESVAFPDWFI ASSKRDQPII
151 LT SELGKSYN TAFELNIND